

PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES
INDIA
1958

VOL. XXVII

SECTION A

PART IV

JULY 1958



NATIONAL ACADEMY OF SCIENCES, INDIA
ALLAHABAD

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PART IV]

SECTION A

[VOL. XXVII

THE INFLUENCE OF CONCENTRATION OF ZINC
IONS ON THE COMPARATIVE ABSORPTION
OF METALLIC IONS BY THE YEAST CELLS
AND YEAST GROWTH

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Received on February 25, 1958

ABSTRACT

Zinc has been shown to influence profoundly the absorption of PO_4''' , Mg^{++} and consumption of nitrogen and sugar by the cells of *Pichia indica*. With the increase of Zn^{++} the consumption of sugar, and phosphate by the yeast cells decreases, whereas the absorption of Mg^{++} increases.

Further, it has been observed that when the maximum yield of yeast cells is obtained, the absorption of mineral nutrients, i.e., Mg^{++} , Ca^{++} , PO_4''' carbon and nitrogen is least.

INTRODUCTION

It has been observed that many micro-organisms can be grown in synthetic media containing minerals, ammonium or nitric nitrogen as the source of nitrogen and carbohydrate as a source of carbon. In these synthetic media the presence of Ca^{++} , Mg^{++} , K^+ , Na^+ , PO_4''' , Cl' and SO_4'' are essential for yeast growth. However, some metallic ions like Fe^{+++} , Mn^{++} , Co^{++} , Cu^{++} and Zn^{++} have profound influence on the metabolic activity of the micro-organism for they disturb the enzymic equilibrium of the complex enzymic systems present in the microbial cells by activating some of the enzymes and inhibiting the activities of some other enzymic systems. This leads to the variation in the products formed by the micro-organism.

Brack¹ observed that in a synthetic medium containing excess of ferrous sulphate a larger quantity of *Penicillium patulum* and a small amount of gentisyl alcohol are formed in presence of Zn^{++} . But when the quantity of ferrous sulphate is only 1 mg./l. the culture indicates the formation of a larger quantity of gentisyl alcohol with a very poor yield of *patulum*.

Chargaff² observed that *E. coli* contains an enzyme which deaminates serine with accumulation of pyruvic acid. Binkley³ found that Zn^{++} acts as activator of this enzyme. Zinc has been found to be essential for growth and subtilin formation by *Bacillus subtilis*.⁴ Foster⁵ suggests that the enzyme systems of micro-organisms and moulds become surfeited and latent reserve enzyme systems are called into play leading to shunt of metabolism in presence of metals.

In this paper we have investigated the influence of Zn^{++} on the intake of PO_4''' , Ca^{++} and Mg^{++} ions by the yeast cells and have also investigated its role on the consumption of nitrogen and sugar by the cells. The organism selected was *Pichia indica*⁶ commonly known as *Dhar yeast*.⁷⁻⁸

EXPERIMENTAL

For the standardisation of A.R. Substances, which were used in the experiments, a blank estimation was done.

Culture media of the following composition was prepared: magnesium carbonate 0.5 gm.; calcium carbonate 0.5 gm.; potassium sulphate 0.5 gm.; sodium chloride 0.5 gm.; disodium hydrogen phosphate 0.5 gm.; ammonium sulphate 6.25 gm. and sucrose 50 gm. The solution of the mineral nutrients were prepared in a flask and its volume raised to 1000 c.c.

In each flask 400 c.c. of the solution was taken and the requisite amount of zinc sulphate was added, adjusting the pH of the culture media to 4.5. The flasks were plugged with non-absorbent cotton and the cultures were sterilized in an autoclave at 15 lb. pressure for half an hour. On cooling the cultures were seeded with a trace of an activated sample of *Dhar yeast*.⁹⁻¹⁰

The period of fermentation was 85 days. The cultures were kept at room temperature which varied between 18° C. and 35° C. Thus initially before fermentation each culture contained 20 gm. of sucrose, 57.8 mgm. of Mg^{++} , 83 mgm. of Ca^{++} , 106 mgm. of PO_4''' and 530 mgm. of nitrogen.

The yeast cells were separated by filtration through weighed filter-papers which were dried at 60° C. for 12 hours and kept in a desiccator overnight. The culture solution containing yeast cells was filtered through the filter-paper and the yeast cells were washed with distilled water and dried in an air-oven at 70° C. to 80° C. for about 8 hours and then placed in a desiccator for cooling. The filter-paper with yeast cells were weighed and the difference of the two weights in each case gave the weight of the dry yeast.

After separating the yeast cells, the culture solution together with washings of the yeast cells was made upto 500 c.c. and this solution was employed for the various estimations. 20 c.c. of this solution were used for the estimation of sugar; 20 c.c. for the estimation of nitrogen and 440 c.c. were used for the estimation of phosphate, calcium and magnesium.

The nitrogen of the culture was estimated by Kjeldahl and Gunning method.

The estimation of sugar was done by the reduction of Fehling solution and employing Methylene blue as indicator near the end-point.

The culture solution was evaporated to nearly dryness and treated with 1 c.c. of concentrated sulphuric acid and evaporated to dryness again. The whole mass was treated with 5 c.c. concentrated nitric acid and again evaporated to dryness. Then the solution was made in a little of concentrated nitric acid and diluted it to 200 c.c. The phosphate of the solution was estimated as phospho-ammonium molybdate, using ammonium molybdate as precipitating agent. After removing phosphate the calcium of the solution was estimated volumetrically by titrating it against standard potassium permanganate solution. After removal of calcium, the solution was concentrated and the magnesium was estimated as magnesium pyrophosphate.

RESULTS

TABLE I

Influence of Zn^{++} on the nitrogen absorption and the yeast growth

Zn^{++} in culture media (mgm.)	Nitrogen absorbed by the yeast cells (mgm.)	Nitrogen absorbed by one gm. of yeast cells (mgm.)	Total weight of yeast cells (gm.)
Nil	515.8	300.9	1.7138
10.1	502.3	288.3	1.7420
40.51	526.5	361.6	1.4560
60.7	526.5	369.7	1.3400

TABLE II

Influence of Zn^{++} concentration on consumption of sugar and yeast yield

Zn^{++} in culture media (mgm.)	Sugar consumed (gm.)	Sugar consumed per gm. of yeast (gm.)	% yield of yeast cells on the basis of sugar consumed
Nil	17.51	10.21	9.21
10.1	16.50	9.47	10.55
40.51	15.63	10.73	9.31
60.7	14.25	10.63	9.40

TABLE III

The absorption of Ca^{++} , Mg^{++} and PO_4''' by the yeast cells at different concentrations of Zn^{++} in the yeast cultures

Zn^{++} added in the culture (mgm.)	PO_4''' consumed (mgm.)	PO_4''' consumed per gm. of yeast cells (mgm.)	Ca^{++} ab- sorbed (mgm.)	Ca^{++} consumed per gm. of yeast cells (mgm.)	Mg^{++} ab- sorbed (mgm.)	Mg^{++} ab- sorbed by one gm. of yeast cells (mgm.)
Nil	12.6	7.40	9.3	5.42	7.6	4.43
10.1	5.6	3.21	0.9	0.51	3.6	2.06
40.51	6.5	4.46	0.9	0.61	12.2	8.37
60.7	1.6	1.19	4.9	3.65	11.2	8.35

DISCUSSION

Increase of Zn^{++} in the culture of *Pichia indica* increases the nitrogen consumption by the yeast. Presence of 10.1 mgm. of Zn^{++} per 400 c.c. of the culture increases the growth of yeast but as the concentration of Zn^{++} is further increased, the yield of yeast decreases.

The presence of Zn^{++} in the culture of the yeast decreases sugar consumption. Increase of the Zn^{++} concentration decreases the sugar consumption but as this is followed by decreased yeast yield the quantity of sugar consumed per gram of yeast grown remains almost constant.

It is interesting to observe that at the concentration of Zn^{++} in the culture which gives the best yield, i.e., 10.1 mgm. of Zn^{++} per 400 c.c. of the culture, least quantities of nitrogen and sugar are consumed.

The presence of Zn^{++} profoundly influences the PO_4''' intake by the yeast cells and increase of Zn^{++} concentration in the culture decreases PO_4''' absorption by the yeast cells. Thus in the culture which does not contain Zn^{++} , 7.4 mgm. of PO_4''' are absorbed for the formation of 1 gm. of yeast cells but if 60.7 mgm. of Zn^{++} are present per 400 c.c. of the culture only 1.6 mgm. of PO_4''' are utilised for the formation of 1 gm. of yeast.

The presence of small concentration of Zn^{++} in the culture greatly decreases the absorption of Ca^{++} by *Pichia indica* cells. But if the quantity of Zn^{++} is greatly increased in the culture, the absorption of Ca^{++} is favoured. It is interesting to note that though Zn^{++} hinders the calcium ion absorption, the absorption of Mg^{++} is increased by the presence of Zn^{++} in the culture and remains constant with the increase of Zn^{++} concentration in the culture.

Above experiments indicate an important fact that at the concentration of Zn^{++} in the culture when maximum yeast yield is obtained, the absorption of phosphate, calcium and magnesium ions is least.

SUMMARY

Presence of small concentration of zinc ions in the culture of *Pichia indica* increases yeast yield but consumption of sugar by the yeast cells decreases with the increase of Zn^{++} concentration in the culture. Absorption of PO_4''' is profoundly decreased by the increase of Zn^{++} in the culture. Calcium absorption is hindered only when the concentration of Zn^{++} remains small in the culture and magnesium ion absorption is increased. Presence of 10.1 mgm. of Zn^{++} per 400 c.c. of the culture is found to be optimum for the yeast growth but at this concentration of Zn^{++} when maximum yield of yeast is obtained, minimum quantities of PO_4''' , Ca^{++} and Mg^{++} are absorbed.

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STABILITY OF THE SOL OF HYDROUS FERRIC OXIDE

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Received on September 27, 1957

INTRODUCTION

THE stability and composition of colloidal particles of hydrous ferric oxide have attracted the attention of numerous workers. Pauli and Valko,¹ Thomas² and others are of the opinion that the particles consist of complex ions allied to Werner's compounds but according to Weiser³ no definite formulæ can be ascribed to them. Britton,⁴ however considered that these hydrous ferric oxides contain some basic salts, but Ghosh⁵ and co-workers have emphasized the role of adsorption of anion by the hydrous oxide. Potentiometric method for the estimation of free chloride in the hydrous oxide dispersed in water was first adopted by Ravinovich and Kargin and later on by Weiser, they found that an increase in concentration of the coagulating electrolyte set free more of chloride from the double layer of the colloidal unit. It is, therefore, expected that the total chloride from the double layer can practically be set free by the addition of a large amount of electrolyte and can thus be potentiometrically estimated. The present paper deals with the composition of colloidal micelles of different grain sizes. The results show that the association of the anion with the hydrous oxide is related to the size of the colloidal particles. It has also been shown that the electrical conductivity of the colloidal micelles rapidly increases with the decrease in the size of the colloidal particles. It is concluded that the colloidal hydrous ferric oxide tends to behave as colloidal electrolytes when the grain size is very small.

Hydrous ferric oxide was precipitated from concentrated solution of ferric chloride (B.D.H.) with the addition of an equivalent amount of ammonium hydroxide, at room temperature with constant stirring. The precipitate was washed by decantation till it tended to peptise. This precipitate was passed through a Sharples Laboratory Supercentrifuge maintained at speed 24,000 r.p.m. Distilled water was constantly fed to the supercentrifuge and the process was continued till the outcoming liquid was found free from chloride. Now the reddish brown hydrous ferric oxide was removed from the bowl, made into a paste with water, using a mortar and pestle, and poured into a large bulk of water for further washing. It was continued till the supernatant liquid was found free from chloride ions. The precipitate of hydrous ferric oxide was peptized, by shaking at a constant rate by a Microid Shaker for about 48 hours, with Hydrochloric Acid (A. R. Quality) of approximately 0.04 N strength. A clear brick red colloidal solution of hydrous ferric oxide was obtained.

By keeping the Sharples Laboratory Supercentrifuge at the speeds of 4,000, 15,000 and 24,000 r.p.m.; three solution samples of different grain sizes were obtained from the same mother solution. The chloride and iron contents of these

four samples were estimated both gravimetrically and volumetrically. For the purpose of study all the four samples were made to contain the same amount of iron per litre of the solution, by diluting wherever necessary. On estimation the chloride content was also found practically to be same in all the four samples.

Nomenclature of the hydrrous ferric oxide sols.—The original sols were termed A and those obtained at 4,000, 15,000 and 24,000 r.p.m. were termed B, C and D respectively. The concentration of iron per litre was 6.14 gm. and 0.07689 gm. atoms as Fe_2O_3 and Fe respectively, while that of chlorine was 0.01748 gm. atoms.

P_H —and conductivity results.—The P_H and conductivity measurements were performed with Cambridge Portable P_H Meter and Leeds Northrup Conductivity apparatus with Audio-Frequency Oscillator respectively. The following results (given in Table I) were obtained.

TABLE I
Temperature 30° C.

Hydrrous ferric oxide sol sample (1)	Hydrogen-ions concentration in		Hydrogen ions associated with colloidal micelles in milligram per litre (4)	Specific conductivity $\times 10^3$ in mhos		Specific conductivity contribution of colloidal micelles in sol sample $(5-6) \times 10^3$ mhos. (7)
	P_H (2)	Milligram ions per litre of solution (3)		of hydrrous ferric oxide sol sample (5)	of hydrochloric acid of P_H corresponding to sol sample in (1) (6)	
A	2.34	4.571	12.909	2.493	1.950	0.543
B	2.36	4.365	13.115	2.432	1.855	0.577
C	2.37	4.266	13.214	2.538	1.807	0.731
D	2.38	4.169	13.311	2.623	1.742	0.881

Hydrochloric acid solutions containing hydrogen ions as given in column (3) of Table I, present in the hydrrous ferric oxide sol samples A, B, C and D were prepared and their specific conductivities are given in column (6), assuming that the total conductance of the colloidal micelles and the acid-free in the sol samples, the specific conductance contributed by colloidal micelles has been obtained from the difference in the values given in columns (5) and (6).

The conductivity values of hydrrous ferric oxide sol samples A, B, C and D and the conductivity values of the colloidal micelles [as shown in column (7)] in the corresponding sol samples have been plotted against the corresponding P_H values as shown in Figs. 1 and 2.

Coagulation of hydrrous ferric oxide sol with KNO_3 , KCl , $\text{K}_2(\text{SO}_4)$ and $\text{K}_3\text{Fe}(\text{CN})_6$.—It is well known that the coagulation values of electrolytes for a solution is highly effected by the time given for the observation. Hence in order to study the stability of different solutions by these electrolytes, first the time of complete coagulation was noted by different concentrations of electrolytes. The

inverse of time of coagulation in seconds and the concentration of the electrolytes are plotted from whence the concentration of the electrolyte necessary for complete coagulation in infinite time, *i.e.*, when $1/t = 0$ has been obtained, and thus eliminating the effect of time on the observations.

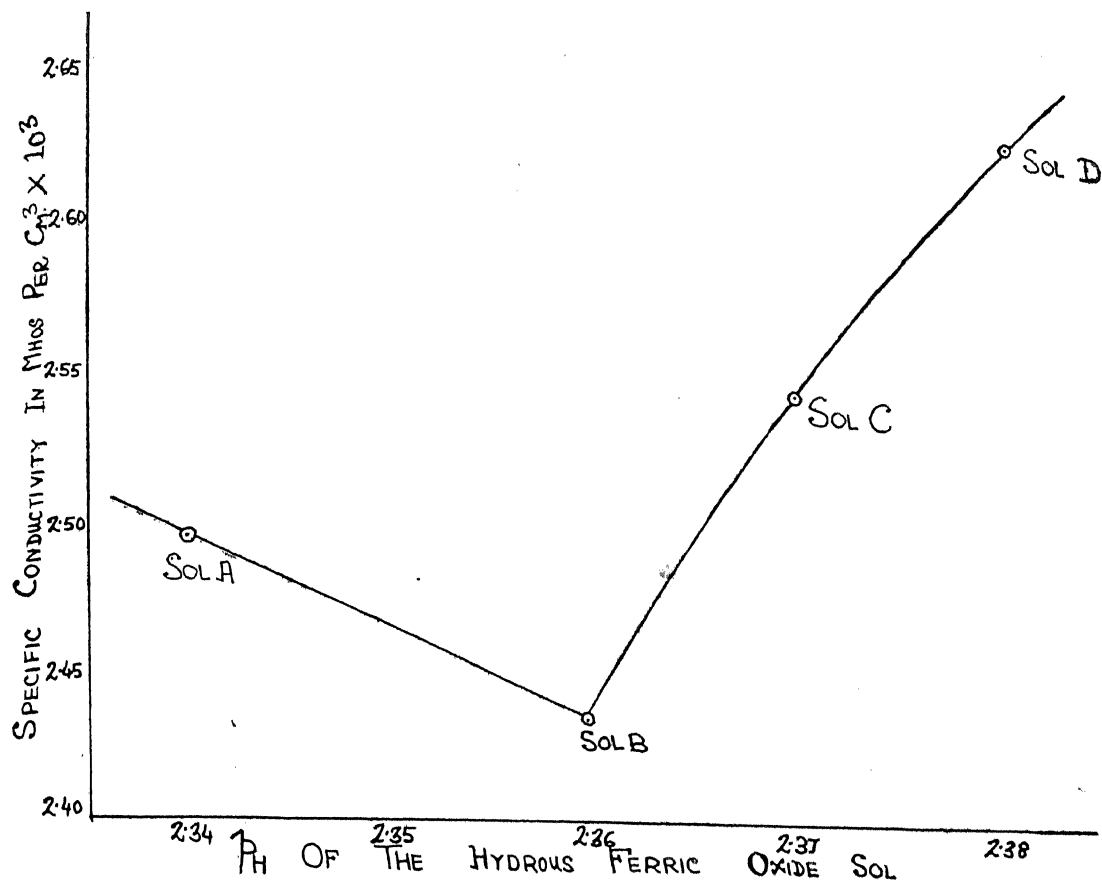


FIG. 1. Specific conductivities of the hydrous ferric oxide sols plotted against their corresponding pH values.

One ml. of hydrous ferric oxide sol was taken in each case of a series of clean and dry test-tubes. Different volumes of a standard solution of electrolyte, diluted with the requisite amount of distilled water, to make the total volume equal to 14.0 ml., were taken in another series of test-tubes. These series of test-tubes were kept in a bath at 35° C. The solution and the electrolyte mixture, whence they attained the temperature of the bath, were mixed together and the time was noted. The time of complete coagulation in each case was noted at the point of visible separation of clear liquid at the upper surface of the test-tube. The electrolytes used were KNO_3 , KCl , K_2SO_4 and $\text{K}_3\text{Fe}(\text{CN})_6$.

The amounts of electrolytes required for complete coagulation, at infinite time were obtained by extrapolation (*vide* Figs. 3, 4, 5 and 6). These concentrations with different electrolytes are given in Table II.

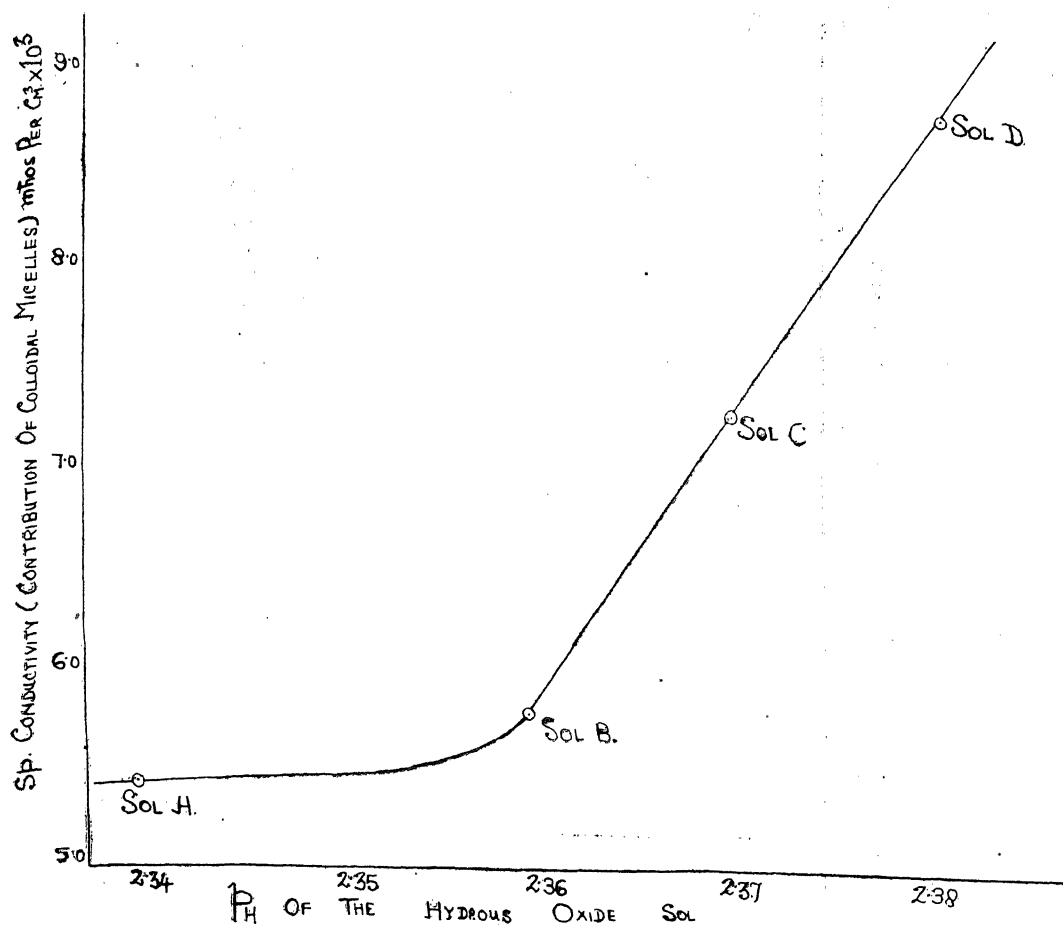


FIG. 2. Conductivity contributions of the colloidal micelles of hydrous ferric oxide sol plotted against their corresponding pH values.

TABLE II

Hydrous ferric oxide sol	Volume of coagulating electrolyte in ml. for complete coagulation at infinite time			
	N KNO_3	N KCl	0.001N K_2SO_4	0.00067 N $\text{K}_3\text{Fe}(\text{CN})_6$
A	1.8	4.0	9.6	12.8
B	2.0	4.25	9.7	12.9
C	2.2	4.50	9.8	13.0
D	2.5	4.50	9.9	13.1

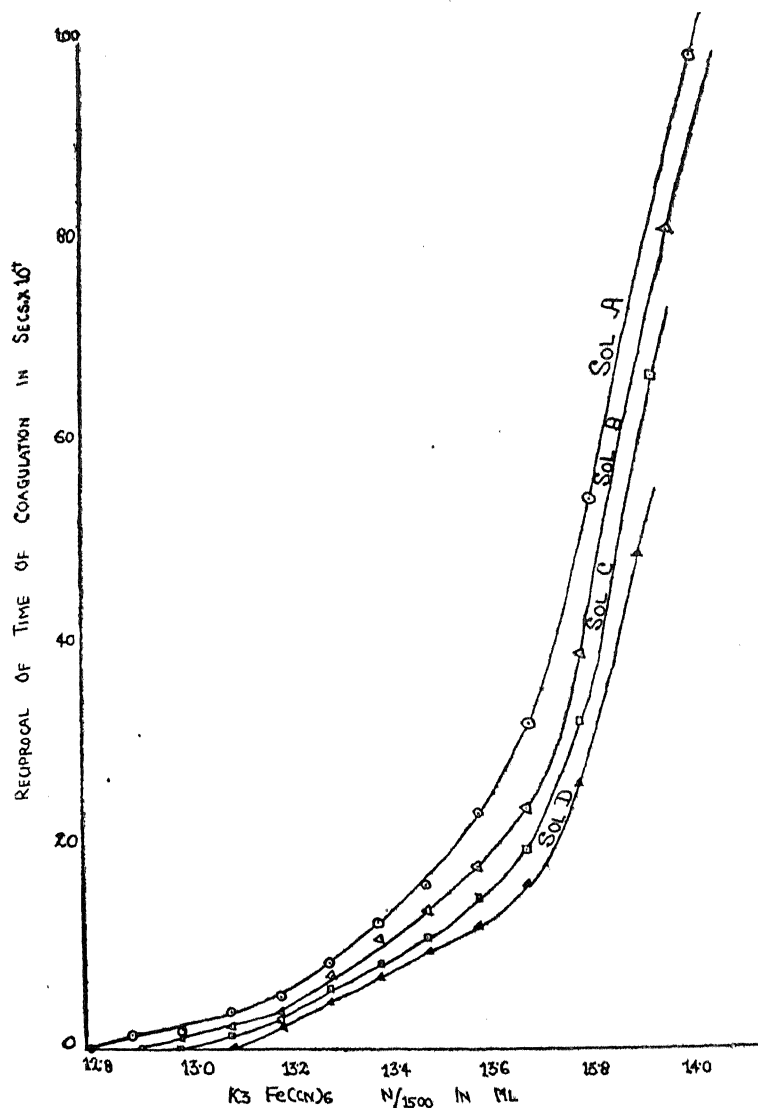


FIG. 3. Reciprocal of time of coagulation plotted against the corresponding volume of $(N/1500) K_3[Fe(CN)_6]$ used for coagulating hydrous ferric oxide sol.

While studying coagulation of hydrous ferric oxide sol with K_2SO_4 as coagulant, under special circumstances the precipitate appeared in layers, separated at regular gaps, similar to the phenomenon observed for Liesegang ring formation. Such appearance of periodic layers of precipitate was specially prominent in the region of slow coagulation of the hydrous ferric oxide sol with K_2SO_4 . It should be emphasized that such periodicity in the precipitation was not observed with any other coagulating electrolyte. The formation of these rings created difficulty in recording the correct time of coagulation. To do away with this difficulty, each tube was shaken at periodic intervals,

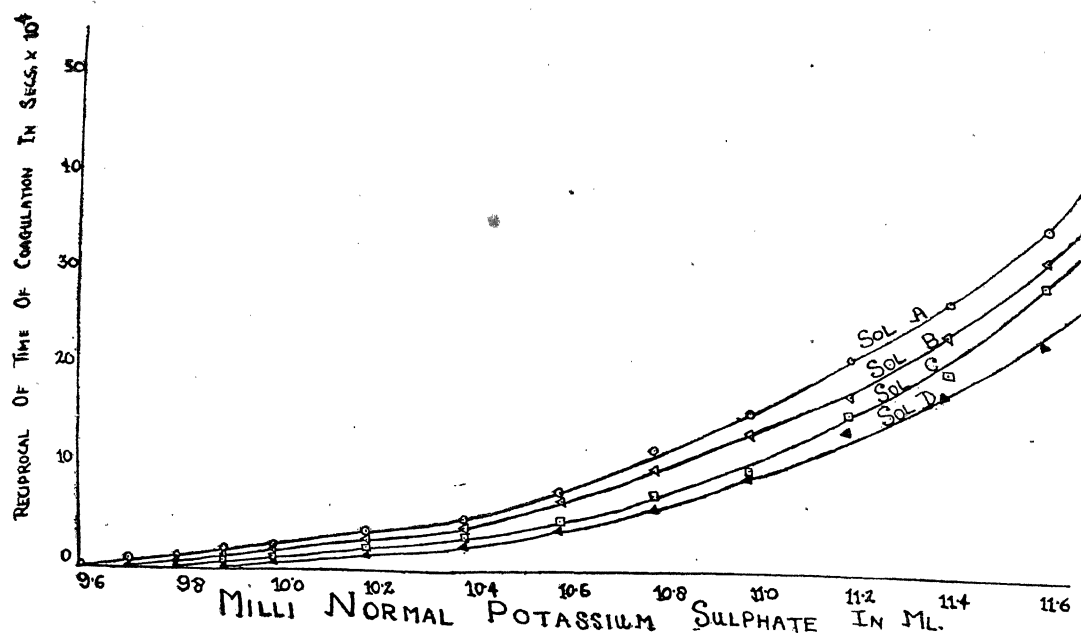


FIG. 4. Reciprocal of time of coagulation plotted against the corresponding volume of Milli normal K_2SO_4 used for coagulating hydrrous ferric oxide sol.

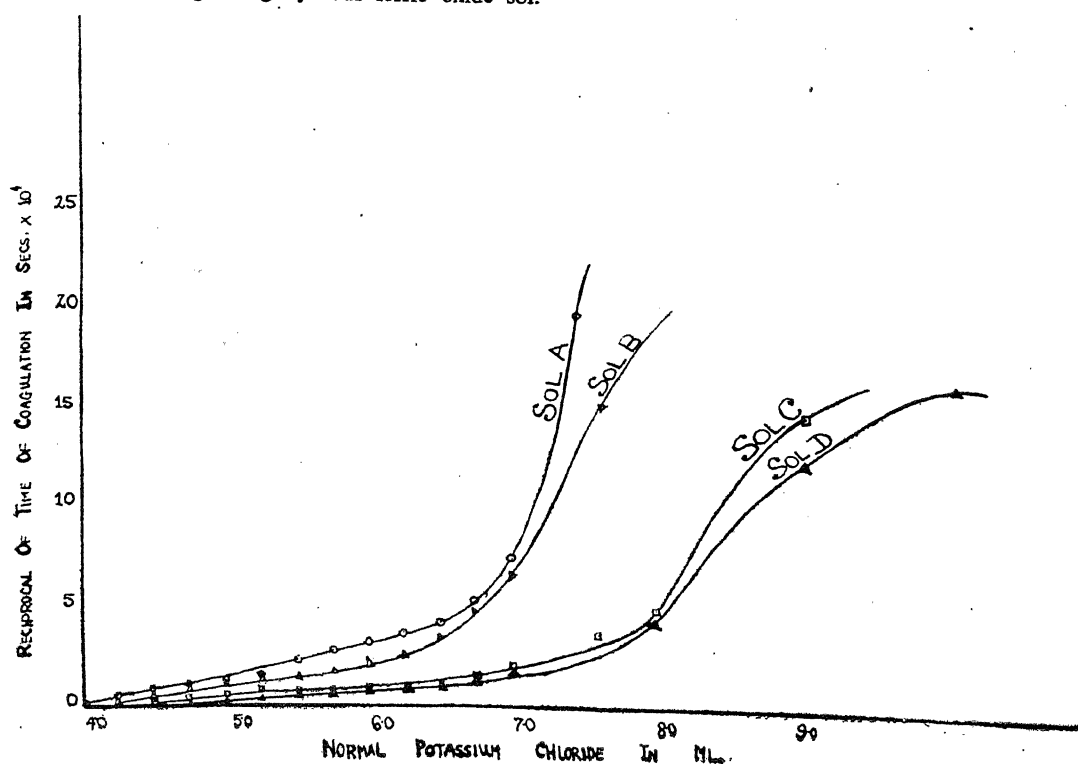


FIG. 5. Reciprocal of time of coagulation plotted against the corresponding volume of normal potassium chloride used for coagulating hydrrous ferric oxide sol.

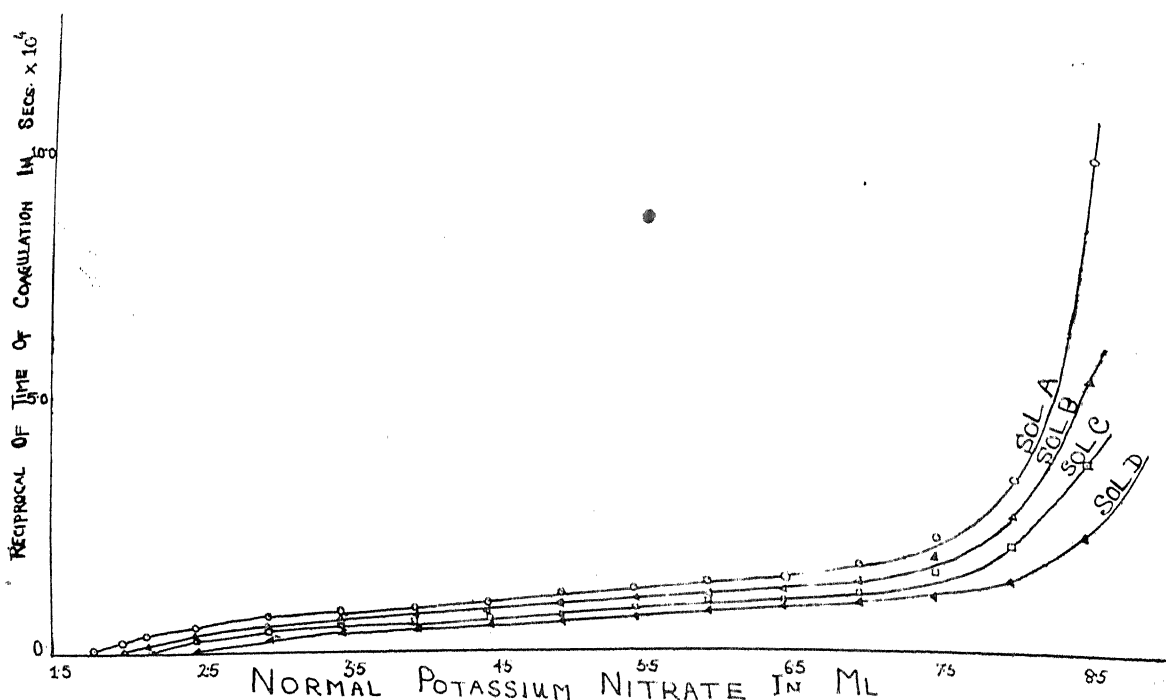


FIG. 6. Reciprocal of time of coagulation plotted against the corresponding volume of normal potassium nitrate used for coagulating hydrous ferric oxide sol.

Determination of the composition of colloidal micelles.—The amount of hydrogen ions associated with the colloidal particles can be estimated from the knowledge of total acid present for obtaining the hydrous ferric oxide sol and the hydrogen ions present free in the sol estimated potentiometrically as shown in Table I. The amount of anion associated with the micelle can be determined by assuming that the chloride bound in the double layer is completely displaced and becomes osmotically active, when the electrical charge on the colloidal particles is removed by the addition of an electrolyte. We are thus able to estimate the chloride-free in the solution and also the chloride bound up in the double layer, the rest of the chloride, of the total chloride is obviously associated with the colloidal micelles.

Estimation of free chloride by potentiometric method.—Similar procedure was adopted, as used by Weiser to estimate the osmotically active chloride in the hydrous ferric oxide sol with the stepwise addition of different electrolytes.

In the potentiometric determination of chloride it was found more convenient to use a half cell tube as calomel electrode. It consists of a Pyrex tubing, having inner diameter 15 mm., connected with a narrow bent tube, ending in a jet to be immersed into the bridge solution (Fig. 7). The narrow bore tube, having a platinum wire fused at the bottom is fixed to a rubber cork, which exactly fits on the top of the wider tube. The platinum wire at the bottom of this tube serves to make contact with mercury kept at the bottom of the wider tube with mercury-calomel paste.

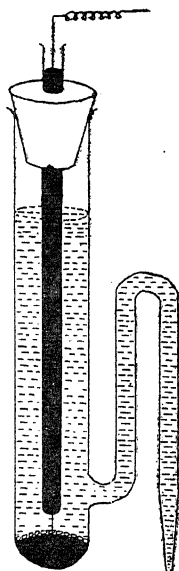


FIG. 7

The pure calomel was suspended in 5 c.c. of each of the samples of hydrous ferric oxide sols A, B, C and D, and kept for 24 hours with occasional shakings. Different amounts of electrolyte diluted to 10 c.c. were added to the hydrous ferric oxide sol, which was saturated with calomel. The whole mixture was transferred into the half cell tube. The half cell tube, containing hydrous ferric oxide sol and electrolyte mixture, was kept standing for an hour. The potential of the resulting half cell was measured against a standard normal calomel electrode with Leeds and Northrup Potentiometer and Multiflex Galvanometer, using saturated solution of ammonium nitrate as bridge solution. A series of these half cells were made with increasing amount of electrolyte. The potential values are given* in Tables III and IV and the electrolytes used with the sols were KNO_3 and K_2SO_4 .

In these observations 5 c.c. of the sols were always diluted to 15.0 c.c. with electrolyte and water, hence the concentration of osmotically active chloride in the original solution sample would be thrice the observed values. Graphs were plotted with the number of ml. of electrolyte used and osmotically active free chloride observed potentiometrically. The graphs obtained by using KNO_3 and K_2SO_4 as coagulating electrolytes, were of S-shape similar to those obtained by Weiser.

The liberation of chloride from the electrical double layer, by the addition of sufficient amount of electrolyte, became practically constant, where we may assume that all the chloride ions in the double layer have been released. In Table V the amount of chloride associated with the micelle is given in column (7).

We are thus in the position to calculate the excess of hydrogen ions adsorbed in excess over the chloride ions adsorbed and thus we obtain the value of the

TABLE III
Potentiometric observations with KNO_3
Temperature 30° C.

Ml. of normal KNO_3	sol A		sol B		sol C		sol D	
	π in volts	Osmotically active chloride in milligram ions per litre	π in volts	Osmotically active chloride in milligram ions per litre	π in volts	Osmotically active chloride in milligram ions per litre	π in volts	Osmotically active chloride in milligram ions per litre
0.0	0.16920	1.530	0.17045	1.458	0.17090	1.433	0.17150	1.401
1.0	0.15775	2.372	0.15914	2.250	0.16020	2.157	0.16125	2.075
2.0	0.15465	2.671	0.15535	2.600	0.15583	2.550	0.15638	2.501
2.5	0.15417	2.721	0.15486	2.651	0.15561	2.575	0.15535	2.601
3.0	0.15388	2.752	0.15438	2.701	0.15475	2.661	0.15520	2.615
3.5	0.15370	2.771	0.15420	2.719	0.15440	2.698	0.15450	2.687
4.0	0.15350	2.786	0.15380	2.760	0.15410	2.729	0.15342	2.718
4.5	0.15290	2.856	0.15301	2.847	0.15320	2.824	0.15340	2.803
5.0	0.15260	2.890	0.15250	2.889	0.15290	2.857	0.15310	2.835
5.5	0.15230	2.905	0.15220	2.904	0.15220	2.901	0.15260	2.890
6.0	0.15201	2.956	0.15201	2.956	0.15210	2.945	0.15210	2.944
6.5	0.15194	2.964	0.15200	2.959	0.15205	2.951	0.15220	2.934

TABLE IV
Potentiometric observations with K_2SO_4
Temperature 30° C.

Ml. of K_2SO_4 N/1000	sol A		sol B		sol C		sol D	
	π in volts	Osmotically active chloride in milligram ions per litre	π in volts	Osmotically active chloride in milligram ions per litre	π in volts	Osmotically active chloride in milligram ions per litre	π in volts	Osmotically active chloride in milligram ions per litre
0.0	0.16920	1.530	0.17045	1.458	0.17090	1.433	0.17150	1.401
0.5	0.16568	1.751	0.16640	1.702	0.16800	1.602	0.16940	1.521
1.0	0.16290	1.949	0.16340	1.910	0.16425	1.849	0.16500	1.801
1.5	0.16020	2.162	0.16060	2.125	0.16155	2.051	0.16210	2.010
2.0	0.15788	2.360	0.15800	2.351	0.15901	2.259	0.15960	2.212
2.5	0.15561	2.575	0.15575	2.563	0.15650	2.489	0.15730	2.413
3.0	0.15342	2.801	0.15392	2.748	0.15523	2.613	0.15530	2.605
3.5	0.14905	3.311	0.14980	3.217	0.15015	3.175	0.15105	3.067
4.0	0.14428	3.975	0.14512	3.850	0.14580	3.751	0.14618	3.695
4.5	0.14270	4.222	0.14300	4.176	0.14340	4.111	0.14365	4.072
5.0	0.14224	4.298	0.14245	4.265	0.14280	4.247	0.14305	4.168
5.5	0.14202	4.335	0.14240	2.272	0.14245	4.264	0.14255	4.247

TABLE V

Hydrous ferric oxide sol	Total iron in milligram atoms per litre	Total chlorine in milligram atoms per litre	Osmotically active chloride in milligram per litre	Chloride ions in double layer and associated with colloidal micelles in milligram ions per litre	Osmotically active chloride at complete coagulation with K_2SO_4 in milligram ions per litre	Displaced chloride from double layer in milligram per litre	Chloride adsorbed on the colloidal micelles in milligram ions per litre	Ratio of per gram atom of iron to gram atom of chlorine adsorbed by the micelle
1	2	3	4	5	6	7	8	9
A ..	76.89	17.48	4.590	12.890	13.005	8.415	4.475	1 : 0.0582
B ..	do.	do.	4.374	13.106	12.816	8.442	4.664	1 : 0.0606
C ..	do.	do.	4.299	13.181	12.792	8.493	4.688	1 : 0.0609
D :	do.	do.	4.203	13.277	12.741	8.538	4.739	1 : 0.0616

TABLE VI

Hydrous ferric oxide sol sample	Total hydrogen ions adsorbed on the colloidal micelles in milligram ions per litre	Total chlorine associated with the colloidal micelles in milligram ions per litre	Positive charge on the colloidal micelles due to hydrogen-ions concentration in milligram ions per litre
(1)	(2)	(3)	(4)
A.	12.910	4.475	8.435
B	13.115	4.664	8.451
C	13.214	4.688	8.526
D	13.311	4.739	8.538

electric charge on the colloidal particles, imparted by the adsorption of hydrogen ions.

DISCUSSION

A perusal of Tables V and VI shows, that the amount of hydrogen ions adsorbed by the hydrous ferric oxide sol by different grain size gradually increases with the decrease in the size of the colloidal particles and the same is

true for the total chloride adsorbed by the colloidal micelles. The ratio of per gram atom of iron to gram atom of chloride, constituting the smaller colloidal micelles varies from 1:0.0582 to 1:0.0616 for bigger colloidal micelles, as shown in column 9 in Table V. In other words, the amount of chloride associated with hydrous ferric oxide, constituting the colloidal micelles, changes with their sizes. Further it may be concluded that small grain size colloidal particles contain greater amount of adsorbed hydrogen ions as shown in column 2 in Table VI, which necessarily takes up greater amount of chloride ions on the colloidal unit itself.

Further it is found that hydrogen ions, imparting electric charge, increase with the decrease in the size of the colloidal grains and it is so because more surface is available for adsorption from smaller size colloidal particles than from bigger ones. The results presented here, therefore conclusively prove that the colloidal micelles carry with them both negative and positive charged ions and the excess of one over the other determines the net electric charge that they carry. It is for the greater electric charge on the smaller size colloidal micelles that they are more stable for electrolytes (*see* Table II). This behaviour is, however, more prominent for monovalent than for the polyvalent electrolyte. As hydrochloric acid is removed by dialysis from a sol of hydrous ferric oxide, the precipitation values for a monovalent anion fall more rapidly than for polyvalent one.

It may be further concluded from the results given here, that the colloidal micelles also contribute to the conductance of the solution and smaller the micelle size, greater the contribution in the electrical conductance, as shown in Fig. 2. The amount of chloride associated on the colloidal micelle, is a function of the surface of the micelles and is associated with colloidal micelles, in order to neutralise the growing charge by the adsorption of hydrogen ions on the colloidal micelles. Therefore the association of anions is not due to the formation of any definite complex compound with hydrous ferric oxide particles as postulated by others.

SUMMARY

The paper deals with the composition of colloidal particles of different grain sizes. The results show that the association of the anion with the hydrous oxide is related to the size of the colloidal particles. It has also been shown that the contribution to electrical conductivity by the colloidal micelles rapidly increases with the decrease in the size of the colloidal particles. It can be concluded that the colloidal hydrous ferric oxide tends to behave as colloidal electrolyte when the grain size is very small.

It is found that hydrogen ions imparting electric charge increase with the decrease in the size of the colloidal grains, and it is so because more surface is available for adsorption from smaller size colloid particles than from bigger ones. The results presented here, conclusively prove that the colloidal micelles carry with them both negative and positive charged ions and the excess of one over the other determines the net electric charge that they carry. It is for the greater electric charge on the smaller size colloidal micelles that they are more stable for electrolytes. This behaviour is, however, more prominent for monovalent than for the polyvalent anions.

The amount of chloride associated with the colloidal micelle is a function of their surface and is associated with colloidal micelles in order to neutralise

the growing charge by the adsorption of hydrogen ions and therefore, the association of anions is not due to the formation of any definite complex compound with hydrous ferric oxide particles as postulated by others.

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STUDIES ON THE HYDRAULIC CONDUCTIVITY THROUGH THE COLUMNS OF 'DUMAT SOILS' OF AGRA DISTRICT BEFORE AND AFTER HUMIFYING THE LOCAL WEEDS UNDER LABORATORY CONDITIONS

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Read at the 26th Annual Session of the Academy held at Muslim
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IN continuation of our previous publications on the velocity of flow of liquids through packed columns (1953, 1954, 1955), it has been observed that the determination of the volume of flow of water in c.c. per unit height per unit time (Vh/t) throws light on the changes in the physical properties of soils treated under laboratory conditions with raw organic matter such as weeds and plants.

The velocity of flow of water through columns packed with soils had engaged the attention as early as 1856. Darcy (1856), Slichter (1897-98) and Zunker (1933) determined the velocity of flow of water through a column of soil by their well-known equations. Darcy showed that the velocity of flow is proportional to the pressure gradient and the area of the cross-section; Slichter (1897-98) developed his formula in which the quantity transmitted in c.c. per second was proportional to the pressure gradient and the square of the mean diameter of soil grains and the cross-sectional area; while Zunker (1933) introduced the factors such as effective surfaces, total pore-space and tension-free pore-space, and the type and arrangement of the particles also to frame his well-known equation. There is sufficient evidence to show that the size, density of packing, hydration of the particles have great effects on permeability. Lutz (1934) observed that the permeability of clay increases as hydration of the particles decreases. This was established by comparing the relative permeabilities of Davidson, Iredell, Putnam and Bentonite colloids and the relative degrees of their hydration. The difference in permeabilities was attributed to the nature of the colloids as also $\text{SiO}_2/\text{R}_2\text{O}_3$ ratio of these systems, which increased in the same manner as the hydration. It was also observed that the nature of the adsorbed ions also affects hydration and permeability. Lutz (1934) reported that hydrogen clays were slightly more permeable than the corresponding calcium saturated clays and this was due to the nature of the adsorbed ions and aggregation of the clay particles. According to the concept of Schumacher (1864) and Zunker (1933) the content of non-capillary or tension-free pores largely determines the percolation rate of uniform soil column. Bodman (1936) observed that permeability is very little influenced by textural differences in soils which have high apparent density greater than 1.4 to 1.5. Decrease in total porosity, however, does not always give a true picture of the diminution of permeability.

The effect of forest cover on the properties of the soil was investigated by Burger (1926) who observed that the infiltration rate depended upon the non-capillary porosity of soil. Baver's (1938) observations indicated that permeability of a soil was related to the shape of pF-moisture curve and the tension of the flex point in the pF-moisture curve was closely related to permeability. It was, therefore, assumed that the percolation rate should vary directly with the amount of larger pores and inversely with the force or tension required to drain through them. The ratio of non-capillary porosity to the pF of the flex is taken as porosity factor and it was observed that the percolation rate increased exponentially with the porosity factor of such widely varying materials as sand, zeolites, clays and soils.

Many workers have carried out their investigations on the flow of water through the soil columns and have considered various factors that may influence hydraulic conductivity through the resistance offered by the porous and non-porous part of the column layers. Schofield (1941) and co-workers have shown from the results of their experiments that the rate of water flow was controlled by the width of the pores and viscosity of water. They also observed that the flow ceased because of the great viscous resistance to movement in their films formed in the layers of the column. Quirk and Schofield (1955) studied the effect of electrolyte concentration on soil permeability and observed that permeability of soil decreased below a certain concentration of the electrolyte which is specific for each ion. An attempt has been made by Lutz (1934) to develop an equation relating to the number and length of highly conducting channels and their contribution to hydraulic conductivity.

Mazurak (1955) and *et al.* determined the rate of flow of water entry into an irrigated chestnut soil under long years of cropping and manurial practice and observed that the maximum rate of water intake per hour was greater with manured than the unmanured soil. His results showed that there was no correlation between water intake and texture in the 5' profile; and the rate of water intake in the surface horizon only up to 10" depth was affected by manurial practices and cropping.

In this paper we present the comparative data on the hydraulic conductivity of the untreated and humified soil samples. The hydraulic conductivity was measured by determining the flow in c.c. per unit height per second ($V_1 h/t$) under constant conditions of temperature, applied tension and hydraulic gradient, length of the column and cross-sectional area of the tube. The humification of the weeds in the soil was carried out at constant moisture level under laboratory conditions in order to obtain more standard values than is possible by field experiments.

EXPERIMENTAL

The weeds were freed from the soil and they were crushed, pulverised and sieved through 100 mesh sieve. The raw organic matter, in this form, was mixed with the soil in the ratio of 20 parts of soil to one part by weight of weeds. The whole quantity was macerated with distilled water and left under the shade to humify. The moisture content of the soil was maintained at 15% by weighing in a Beringer's balance on alternate days and adding required amount of distilled water. At the end of sixteen weeks soil samples were taken out and air dried. The soil samples thus obtained were properly sieved (100 mesh). All the experiments were performed in four replicates. The average of the four replicates is given in the tables. Inorganic constituents of the weeds were estimated by the

methods suggested by A.O.A.C. (1945). Porosity was determined by the method suggested by Knowles and Watkins (1947) and cation exchange capacity was determined by the method given by Piper (1947). The method of measuring the velocity of flow of the water through the soil columns was the same as described in our previous communication (1953).

TABLE I
Analysis of weeds added for humification

	Leguminous			Non-leguminous		
	<i>Melilotus indica</i> (Zoonzhru)	<i>Cassia obtusifolia</i> (Chakwad)	<i>Pongamia glabra</i> (Kanji)	<i>Justicia adhotada</i> (Arousa)	<i>Xanthium Strumarium</i> (Chhotta Dahatura)	<i>Cenchrus ciliaris</i> (Anjana)
	%	%	%	%	%	%
Loss on Ignition	85.41	83.06	85.64	88.86	80.69	49.80
Sand ..	4.246	3.659	2.930	1.245	8.523	38.17
Alkali soluble Silica	1.5960	0.357	2.191	1.036	1.379	4.353
Fe ₂ O ₃ ..	0.511	0.144	0.040	0.1570	0.3120	0.858
CaO ..	3.537	3.116	2.073	2.615	3.063	0.938
MgO ..	0.775	0.499	1.108	0.708	0.789	0.609
Al ₂ O ₃ ..	1.593	5.308	2.067	1.385	3.287	1.223
K ₂ O ..	1.8170	1.832	2.846	2.492	4.388	1.207
P ₂ O ₅ ..	0.349	0.420	0.576	0.492	0.461	0.276
$\frac{\text{SiO}_2}{\text{R}_2\text{O}_3}$ ratio ..	5.173	1.265	4.173	2.615	3.373	41.04

DISCUSSION

The data in Table II will show that the values of the volumes of water in c.c. which flowed out through one centimeter of height per second (V_1h/t) are appreciably greater in the untreated soil samples than those of the soil humified with leguminous and non-leguminous weeds and plants for a period of sixteen weeks. These observations evidently suggest that humification of weeds and plants in the soil reduces the hydraulic conductivity of the soil layers made by artificial packing.

Movement of water in soils is a very complicated function of so many factors. The simplest conception of hydraulic conductivity is based on the size and continuity of pores containing the water under constant tension and hydraulic gradient

and on the viscosity of water. The pressure head should be enough to break the air-water meniscus films so that it may allow the drops to come out. According to Poiseuille's equation we can visualise that water will move through soils at an appreciable rate when pores are wide and the rate of flow will be very much reduced by slight alteration in the radius of the pores.

The soil column may be considered to be constituted of numerous capillaries of varying dimensions. The retention of water in the soil has been viewed by Briggs (1897) and others as a function of tension of water films around the particles, while Buckingham (1907) developed the idea of capillary potential to explain the retentivity of soils moisture. But since our data is on the rate of flow of water through porous columns of saturated soil, the interpretation of our results should be mainly based on the non-capillary and capillary porosity, under constant applied tension, hydraulic gradient, viscosity, and density and such other factors as may not be directly determined. Baver's (1938) experimental results have already shown that the percolation rate increases exponentially with the porosity factor which depends upon the ratio of non-capillary porosity to pF of the flex in pF-moisture curves.

Yet the fact remains that the hydraulic conductivity or the rate of flow through the layers of the soil column packed in a tube may also be affected by the undetermined physico-chemical forces of the soil colloids. It is well known that silica is a strongly polar substance and so is the soil humus complex. On account of the polar nature of the system the exchangeability of the ions is a fundamental property of the soil particles. Water is a polar compound and hence it may be visualised that there is a possibility of the orienting forces working on the molecules of water or any other polar liquid which may be made to flow through the capillaries of the soil.

Studies on hygroscopicity, swelling, viscosity, dispersity of clays lead to the generalisation that the orientation of adsorbed water molecules on the surface of the colloidal clay particles takes place during hydration. The nature of the crystal lattice, chemical composition of the clay mineral, the amount and nature of adsorbed cations also influence the hydration and swelling of the clays. Hence it may be visualised that hydraulic conductivity or rate of flow of water through the soil column must be a very complicated function of the physico-chemical forces which exert an orienting effect on the liquid molecules.

In view of the foregoing physical and chemical influences which prevail in the system it becomes necessary to suggest that the capillary and non-capillary flow of liquids may, in all probability, be influenced by the surface forces which exist in the layers of the soil column. It has been evidenced by different methods that the surface characteristics of soils change during the process of humification. Indications of the orientation effect of molecules of different polar liquids have been obtained in our previous work (*loc. cit.*) when the liquids were made to flow through columns of Silica, Alumina, Calcium carbonate, etc., under identical conditions of the height of the column, applied tension, hydraulic gradient and temperature.

The data given in Table II shows that, although the porosity is increased by humification of weeds, V_1h/t (volume in c.c./unit height/unit time) decreases. It will be seen that in all cases the velocity of flow is less after humification, although the porosity and moisture content of the humified soils are comparatively high. The increase in porosity does not, however, give any quantitative idea of the

variations in the capillary and non-capillary pores. If it could be assumed that the number of non-capillary pores are reduced in the columns packed with humified soils, the decrease in the velocity of flow should of course, be explained by the Poiseuille's equation; but since the surface activity of the particles cannot be completely ignored it is very probable that both these factors are responsible for the decrease of the hydraulic conductivity of the humified soil columns. By the humification of weeds the surface of the colloidal clay complex becomes more active with the result that the water molecules suffer orientation at the surface. Hence, the change in the porosity of the columns supplemented by the orienting force of the surface, seems to determine the velocity of flow.

Interesting support to the above is obtained in the hydraulic conductivity through the soil column humified with *Cenchrus ciliaris* (Anjana grass). In this the $\text{SiO}_2/\text{R}_2\text{O}_3$ was very high as compared to the other weeds and plants. The observed value of V_1h/t with this weed was the lowest. SiO_2 is polar in character and undergoes excessive hydration. Hence it can be concluded that the least hydraulic conductivity shown by the soil humified with Anjana grass is due to the considerable decrease of non-capillary pores and the attraction of the surface for the water molecules flowing through the capillaries.

It is further observed that total cation exchange capacity increased in the treated soils. Since exchangeable cations influence the permeability of soils (Lutz, 1934) it adds further support to the part played by the surface activity of the humified columns in reducing the hydraulic conductivity. Mazurak's (1955) observations, do not, however, correspond to the laboratory conditions under which the velocity of flow has been measured by us. The long cropping and manuring practice might have brought about many other changes which increased the rate of infiltration.

SUMMARY

Hydraulic conductivity through the columns of Dumat soils of Agra District before and after humifying the local weeds under laboratory conditions has been studied. It is interesting to note that the humification of weeds in soils reduces hydraulic conductivity of the soils columns.

It is suggested that the surface characteristics of soils change after humification of the weeds. The lowering of the hydraulic conductivity or velocity of flow of water through the humified soil columns has been explained as being due to the changes in the porosity and physico-chemical properties. It is further suggested that the rate of flow may be influenced by the orientation suffered by the water molecules as they pass through the column layers and capillaries.

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EFFECT OF WHEAT-STRAW ON AVAILABLE NITROGEN IN SOIL IN PRESENCE OF PHOSPHATE

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Received on August 4, 1958

It is a remarkable fact that an acre of soil may contain several thousand pounds of nitrogen in organic combination, yet a dressing of nitrate of soda or sulphate of ammonia, giving 15-20 lb. of nitrogen, often produces an appreciable increase in the crop yields. It is obvious that, by far, the greatest proportion of soil nitrogen is not immediately available for the use of plants. The soils of temperate region are richer in nitrogen content than the soils of tropical countries.¹ This is due to the fact that the prevailing high temperature and intensity of sunlight accelerate the oxidation of carbonaceous and nitrogenous compounds in the tropical soils, thus entailing a loss of soil nitrogen produced by the nitrification of proteins and other nitrogenous compounds. There will be a marked decline in the tendency of the rising available nitrogen content with cultivation.² But the addition of organic matter to the soil or keeping the land fallow raises the nitrogen status (both fixed and available) again, due to two factors operating together, *i.e.*, fixation of atmospheric nitrogen and its conservation in the soil. Thus, of the multifold values of organic matter, its function to govern the nitrogen status of soils in tropical countries has been an important one.

The experiments of Dhar and co-workers³ lead to explain as to why the unmanured soils of the tropics give a better crop yield than the soils of the temperate country under similar comparable conditions. It has been calculated⁴ that an acre of cold country soil approximately contains 2,250-4,500 lb. of total nitrogen per acre of land while in Indian soils, the value is approximately half. On the contrary, the amount of available nitrogen in the cold countries is only 22-45 lb. per acre, this being about 100 lb. more in Indian soils. Bear⁵ has attributed the rate of nitrate accumulation in soils largely to the recentness of the origin of organic materials, temperature and the water content of the soil.

The above observations go to show an important bearing on the Indian agriculture where constant yields are drawn from fields year after year, though they are neither properly manured, nor legumes are grown in them, nor fallowing is customary.

In view of the above considerations, we have studied in this paper, the available nitrogen status of the soil in presence of wheat straw as a conservator as such and also in the presence of di-sodium hydrogen phosphate. The soil samples have been exposed to sunlight and analysed periodically.

EXPERIMENTAL

Soil samples were prepared by powdering the soil under investigation and taking them in pound weights in glass jars. Weighed quantities of di-sodium hydrogen phosphate were added in the form of solution to a set of the jars. Finely powdered wheat-straw was added in weighed quantities to the jars, both containing

phosphate or otherwise. The contents were thoroughly mixed and exposed to sunlight for about eight hours every day. The moisture content was kept approximately constant.

The average temperature during the exposure was 36.4°C .

The soil was analysed⁶ initially and then after 150 days of exposure.

A blank set of similar weight of soil was also exposed under similar conditions without mixing wheat-straw and/or phosphate.

OBSERVATIONS

Analysis of soil:

Total carbon in gm. per cent.	1.8557
Total nitrogen	0.2026
Ammoniacal nitrogen	0.0025
Nitrate nitrogen	0.0042
Moisture content at $100-105^{\circ}$	2.0562
Loss on ignition	6.1245
Silica (SiO_2)	36.0127
Calcium (CaO)	3.3406
Potassium (K_2O)	0.6925
Phosphorus (P_2O_5)	0.4006

Analysis of wheat-straw:

Total nitrogen	0.6371%
Total carbon	39.4721%
Moisture content	4.2272%
Loss on ignition	90.9235%
Ash content	8.0158%
Silica	6.9972%
Calcium (CaO)	0.2898%
Magnesium (MgO)	0.0970%
Potassium (K_2O)	0.0705%
Phosphorus (P_2O_5)	0.0621%

TABLE I

With 1 lb. of soil as such, exposed to sunlight

Period of exposure	Total nitrogen	Total carbon	Carbon oxidized	Nitrate nitrogen	Ammoniacal nitrogen	Total available nitrogen	Available/total nitrogen when total = 100
Original ..	0.2026	1.8557	..	0.0042	0.0029	0.0067	3.31
150 days ..	0.2218	1.4153	0.4404	0.0036	0.0050	0.0081	3.65

TABLE II

With 1 gm. of wheat-straw per lb. of soil

Period of exposure	Total nitrogen	Total carbon	Carbon oxidized	Nitrate nitrogen	Ammoniacal nitrogen	Total available nitrogen	Available/total nitrogen when total = 100
Original ..	0.2040	1.9425	..	0.0042	0.0025	0.0067	3.28
150 days ..	0.2652	1.4510	0.4915	0.0041	0.0055	0.0096	3.62

TABLE III

With 1 gm. of wheat-straw + 1 gm. of phosphate per lb. of soil

Period of exposure	Total nitrogen	Total carbon	Carbon oxidized	Nitrate nitrogen	Ammoniacal nitrogen	Total available nitrogen	Available/total nitrogen when total = 100
Original ..	0.2040	1.9423	..	0.0042	0.0025	0.0067	3.28
150 days ..	0.2743	1.3560	0.5863	0.0043	0.0061	0.0104	3.79

TABLE IV

With 1 gm. of wheat-straw + 3 gm. of phosphate per lb. of soil

Period of exposure	Total nitrogen	Total carbon	Carbon oxidized	Nitrate nitrogen	Ammoniacal nitrogen	Total available nitrogen	Available/total nitrogen when total = 100
Original ..	0.2040	1.9419	..	0.0042	0.0025	0.0067	3.28
150 days ..	0.2849	1.2995	0.6424	0.0042	0.0058	0.0100	3.51

TABLE V

With 1 gm. of wheat straw + 5 gm. of phosphate per lb. of straw

Period of exposure	Total nitrogen	Total carbon	Carbon oxidized	Nitrate nitrogen	Ammoniacal nitrogen	Total available nitrogen	Available/total nitrogen when total = 100
Original ..	0.2040	1.9416	..	0.0042	0.0025	0.0067	3.28
150 days ..	0.2909	1.2365	0.7051	0.0050	0.0058	0.0108	3.71

TABLE VI

With 3 gm. of wheat-straw per lb. of soil

Period of exposure	Total nitrogen	Total carbon	Carbon oxidized	Nitrate nitrogen	Ammoniacal nitrogen	Total available nitrogen	Available/total nitrogen when total = 100
Original ..	0.2068	2.1147	..	0.0042	0.0025	0.0067	3.24
150 days ..	0.2997	1.6150	0.4997	0.0054	0.0063	0.0117	3.90

TABLE VII

With 3 gm. of wheat-straw + 1 gm. of phosphate per lb. of soil

Period of exposure	Total nitrogen	Total carbon	Carbon oxidized	Nitrate nitrogen	Ammoniacal nitrogen	Total available nitrogen	Available/total nitrogen when total = 100
Original ..	0.2068	2.1145	..	0.0042	0.0025	0.0067	3.24
150 days ..	0.3036	1.4342	0.6803	0.0059	0.0064	0.0123	4.05

TABLE VIII

With 3 gm. of wheat straw + 3 gm. of phosphate per lb. of soil

Period of exposure	Total nitrogen	Total carbon	Carbon oxidized	Nitrate nitrogen	Ammoniacal nitrogen	Total available nitrogen	Available/total nitrogen when total = 100
Original ..	0.2068	2.1122	..	0.0042	0.0025	0.0067	3.24
150 days ..	0.3103	1.3919	0.7203	0.0060	0.0062	0.0122	3.93

TABLE IX

With 3 gm. of wheat-straw + 5 gm. of phosphate per lb. of soil

Period of exposure	Total nitrogen	Total carbon	Carbon oxidized	Nitrate nitrogen	Ammoniacal nitrogen	Total available nitrogen	Available/total nitrogen when total = 100
Original ..	0.2067	2.1122	..	0.0042	0.0025	0.0067	3.24
150 days ..	0.3148	1.2815	0.8307	0.0058	0.0064	0.0122	3.88

TABLE X

With 5 gm. of wheat-straw per lb. of soil

Period of exposure	Total nitrogen	Total carbon	Carbon oxidized	Nitrate nitrogen	Ammoniacal nitrogen	Total available nitrogen	Available/total nitrogen when total = 100
Original ..	0.2095	2.2841	..	0.0042	0.0025	0.0067	3.20
150 days ..	0.3221	2.2339	0.6502	0.0057	0.0069	0.0126	3.91

TABLE XI

With 5 gm. wheat-straw + 1 gm. phosphate per lb. of soil

Period of exposure	Total nitrogen	Total carbon	Carbon oxidized	Nitrate nitrogen	Ammoniacal nitrogen	Total available nitrogen	Available/total nitrogen when total = 100
Original ..	0.2095	2.2838	..	0.0042	0.0025	0.0067	3.20
150 days ..	0.3259	1.5075	0.7763	0.0058	0.0072	0.0130	3.99

TABLE XII

With 5 gm. wheat-straw + 3 gm. phosphate per lb. of soil

Period of exposure	Total nitrogen	Total carbon	Carbon oxidized	Nitrate nitrogen	Ammoniacal nitrogen	Total available nitrogen	Available/total nitrogen when total = 100
Original ..	0.2095	2.2832	..	0.0042	0.0025	0.0067	3.20
150 days ..	0.3318	1.4827	0.8005	0.0058	0.0063	0.0119	3.59

TABLE XIII

With 5 gm. wheat-straw + 5 gm. phosphate per lb. of soil

Period of exposure	Total nitrogen	Total carbon	Carbon oxidized	Nitrate nitrogen	Ammoniacal nitrogen	Total available nitrogen	Available/total nitrogen when total = 100
Original ..	0.2095	2.2814	..	0.0042	0.0025	0.0067	3.20
150 days ..	0.3431	1.2895	0.9919	0.0060	0.0070	0.0130	3.79

It has been already shown that the rate of nitrate production in the surface soil depends, both on the amount of nitrogen present in the readily oxidizable organic matter of the soil as well as on the actual rate of oxidation, the latter being rapid in the well aerated soils.⁷ The soil under investigation, when mixed with powdered wheat-straw, with and without phosphate, and after 150 days of exposure to sunlight under properly aerated conditions, gave very interesting results, so far as the available nitrogen of the soil was concerned. The available nitrogen, *i.e.*, the sum of nitrate and ammoniacal nitrogen of the soil, rich in humus, which was 3.31% of the total nitrogen in the beginning of the experiments, showed a considerable increase not only in the total available nitrogen, but also in the total nitrogen at the end of the experiments.

The results go to show that on addition of wheat-straw to soil in the form of a conservator, there is a marked increase both in the available nitrogen and the total nitrogen fixed (Tables I and II). This occurs with a consequent loss of the carbon in the system, showing thereby that the increase of the nitrogen in the system has taken place at the expense of the carbon present therein. It is further to be noticed that with the increasing quantities of wheat-straw in the soil higher percentages of both the total available and fixed nitrogen (Tables II, VI and X) are observed. The higher percentages of nitrogen in the system also show a correspondingly increasing loss of carbon from the soil. When phosphate is added to the soil in presence of wheat-straw, the loss of carbon from the soil becomes well marked (Tables III-V, VII-IX and XI-XIII), this being higher with the increasing doses of phosphate.

The results conclusively show that the percentage of total available nitrogen (available nitrogen/total nitrogen, when total nitrogen = 100) has a declining tendency with the increasing quantities of phosphate doses. In the case of the soils containing 5 gm. of phosphate per lb. of the soil (Tables V, IX, XIII) this percentage shows a slight increase, supposingly due to the increased alkalinity in the system which may cause some of the fixed nitrogen to be transformed into ammonia gas.

Extensive work in this line⁸ has shown that the percentage of available nitrogen in case of soluble carbohydrates and ammoniacal nitrogen in presence of cellulosic substances is considerably increased. But a reverse condition is observed with respect to the total nitrogen, *i.e.*, fixed nitrogen is more with the insoluble wheat-straw or lignite than with the soluble carbohydrates in the soil. The phosphate added to soil, not only acts as a surface catalyst to promote the rate of oxidation of carbon in the soil, but is also utilized by the soil bacteria in building up of their

cells. The cellulose of wheat-straw is not soluble in water like soluble carbohydrates and hence the oxidation of the system does not start so readily and vigorously as with soluble carbohydrates.⁹ Since the fixation of nitrogen and its subsequent conversion into nitrate, which is the first product of oxidation, are caused by the release of energy in the system, brought about by the oxidation of carbon present therein, a higher percentage of fixed nitrogen with a lower degree of available nitrogen are observed. These results are further corroborated by the findings of Bose¹⁰ who reports an increase of 14% and 7.3% available nitrogen with 2% in sugar candy and 2% wheat-straw respectively after 100 days' exposure of soil to sunlight. It has been observed that the soil bacteria prefer to assimilate the nitrate supplied from outside, rather than generating it in the system from the nitrogen and oxygen of the air. In case of wheat-straw it appears that the available nitrogen in the soil is partially taken up by the increased number of micro-organisms for building up of the microbial proteins. Since the velocity of the oxidation of wheat-straw is slow, the nitrification of proteins and consequently the percentage of available nitrogen will be recorded somewhat low. Moreover, part of the available nitrogen may also remain adsorbed, not only on the soil surface, but also on the unoxidized cellulose and carbon which are insoluble in water. The above considerations may put a suitable explanation to the lower percentage of the total available nitrogen, i.e., the ratio of the available to the total nitrogen, the latter being 100.

The details of the process and mechanism of the nitrogen fixation can be suitably explained by the photochemical theory,¹¹ which not coming within the scope of this paper, have not been included.

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SUITABILITY OF CROPS UNDER WATER-LOGGED CONDITIONS

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Received on May 27, 1958

A WATER-LOGGED area is that in which the sub-soil water-table is within 5 ft. from the surface.

There has been a steady rise in the sub-soil in the Punjab. Table I compares the areas having water-table from (0-5 ft.), (5-10 ft.) and (10-15 ft.) on different canals.

TABLE I

Showing the areas having water-table from (0-5 ft.), (5-10 ft.) and (10-15 ft.) depth on different canals (in acres)

Sl. No.	Name of the canal	October (0-5 ft.)		October (5-10 ft.)		October (10-15 ft.)	
		1953	1956	1953	1956	1953	1956
1	Upper Bari Doab Canal	186778	933274	584909	462336	293683	188314
2	Bist Doab Tract	55296	420557	309657	500736	310886	277402
3	Ferozepur Circle	274022	561562	111820	203981	29491	49150
4	Sirhind and Grey Canals	135168	777451	892519	1830909	1282867	1257062
5	Western Jumna Canal	100761	618701	497664	909312	444825	556032
6	Agra Canal Tract in Gurgaon Dist.	Not available	44339	Not available	188634	Not available	138700

The menace of water-logging has not only affected considerably the State of Punjab, but also other States. This has adversely affected the agricultural production of our country. Therefore it was considered necessary to study the type of crop rotation which could be successfully grown on such types of soils. This investigation deals with the different crops tried on these soils within two years.

EXPERIMENTAL

Five pucca tanks 9 ft. \times 5 ft. \times 5 ft. were constructed. They were made impervious by spraying a thin layer of Bitumen inside the tanks. Water-table was

maintained at 1 ft., 2 ft., 3 ft., 4 ft. and 4 ft. 9 in. depth. Small size gravel and sand were placed at the bottom of the tanks. Soil of the following composition was compacted at 1.5 dry bulk density and 11.0% moisture content.

Clay (particles below .002 mm.)	..	15.5%
Silt (particles greater than .002 mm. but less than .02 mm.)	..	26.7%
Sand (particles greater than .02 mm. but less than 2.0 mm.)	..	57.8%
Total soluble salt %	0.12
pH	8.20
Exchangeable calcium in m.e./100 gm. soil	..	11.60
Exchangeable sodium plus potassium in m.e./100 gm. soil	..	2.35

Holes were provided at the sides for recording the water-table in the morning and evening and for adding or taking out water in order to maintain the desired water-tables.

The following rotations was tried in all the tanks.

Wheat, Cotton, Senji (*Mellotus parviflora*) sugarcane.

DISCUSSION OF RESULTS

Wheat was sown in the first week of November after giving the following irrigations to the plots:

Sl. No.	Water-table from surface (in feet)	First irrigation given (in inches)
1	1.0	Nil
2	2.0	2.0
3	3.0	3.0
4	4.0	4.0
5	4.9	3.0

No further surface irrigation was applied to the plots (Nos. 1-3) even upto the maturity of crops. In the case of plot No. 4 (water-table 4.0 ft.) and plot No. 5 (water-table 4 ft. 9 in.), 2 in. and 3 in. irrigations were given in the last week of March. Throughout the above period, the water-table was maintained at the desired depth by adding water from the holes, provided at the sides, Table II

shows the comparative amounts of water added to the subsoil for maintaining the desired water-tables:

TABLE II

Comparison of total amounts of water added for maintaining the desired water-tables sown under different crops

Sl. No.	Depth of water-table (in feet)	Amounts of water added (in inches)			
		Wheat 591	Cotton 320 F	Senji	Sugarcane Co. 290
Rainfall		5.95	19.26	0.74	28.17
1	1.0	18.0	32.0	17.12	71.32
2	2.0	13.5	31.0	16.56	70.87
3	3.0	11.0	28.75	14.58	62.56
4	4.0	7.0	23.75	11.06	52.29
5	4.9	2.75	16.75	10.27	47.30

The yields of wheat are given in Table III.

TABLE III

Yield of wheat in different plots

Sl. No.	Depth of water-table (in feet)	Rainfall (in inches)	Total of rainfall and surface irrigation (in inches)	Yield (in lb./acre)
1	1.0	5.95	5.95	2327.8
2	2.0	5.95	7.95	2480.5
3	3.0	5.95	8.95	2480.5
4	4.0	5.95	10.95	2480.5
5	4.9	5.95	11.95	2347.5

Due to the heavy storm in the month of March, lodging of wheat took place in the plots No. 4 and 5, which affected the yield adversely. There was an attack of rust also:

It was inferred from the above results that wheat could be safely grown in the water-logged areas having water-table even at one foot depth from the natural surface without giving any irrigation. One initial irrigation of 2-3 in. was needed in the case of areas having water-table from 2 ft.-5 ft. depth. One irrigation of 2-3 in. also seemed necessary in March for getting maximum yields in areas having water-table from 4 ft. to about 5 ft. depth, provided there was no rain in this month.

Cotton.—After taking wheat crop, farm-yard manure at 14 tons per acre was applied to all the plots. It was thoroughly mixed with the soil. Cotton 320 F variety was sown in all the plots. Initial irrigation of 1 in., 2 in., 3 in., 4 in. and 4 in. was given to the plots having water-table at 1 ft., 2 ft., 3 ft., 4 ft. and 4 ft. 9 in. respectively. Afterwards no surface irrigation was given, but water was regularly added at the bottom to keep the desired water-table in all the plots (Table II).

In the early stages, the growth of cotton was very good in all the plots. But with the commencement of the monsoon there was sudden rise in the water-table. Though sub-soil water was removed from all the plots for maintaining the desired water-table, yet the excessive moisture content in the profiles affected the plots adversely. The plants remained stunted in the first, second and third plots, where the average heights were 38.0 in., 44 in., 25 in. and 48.75 in. respectively. The average heights in the fourth and fifth plots were 60 in. and 64.4 in. respectively.

The heavy rains in the last week of September and early October again damaged the plants considerably even in the fourth and fifth plots. The yields of cotton in the different plots are given in Table IV.

TABLE IV
Yield of cotton in different plots

Sl. No.	Depth of water-table (in feet)	Rainfall (in inches)	Total of rainfall and surface irrigation (in inches)	Yield (in lb./acre)
1	1	17.26	18.26	618
2	2	17.26	19.26	943
3	3	17.26	20.26	1240
4	4	17.26	21.26	1736
5	4-9	17.26	21.26	1859

The following broad inferences were drawn.

(a) Cotton being a long root crop did not grow well in areas having water-table upto 3 ft. depth. There was a bad opening of the balls and the lint was also defective.

(b) The lint of cotton, sown in areas of 4 ft. depth water-table was also not of the proper strength. Provision of drains might avoid the failure to some extent.

(c) Normal yields can be expected from cotton sown in areas having water-table at about 5 ft. depth.

Senji (*Mellotus parviflora*).—Cotton was followed by *Senji*. One in. irrigation was given initially to all the plots. Surface irrigation of 3.0 in. was applied to the plots No. 3, 4 and 5. The total rainfall during the period was 0.74 in.

In the beginning, the crop was poor in plots No. 1, 2 and 3. It was best in plot No. 5. With the approach of spring season, *i.e.*, after 15th February, the crop improved in all the plots. The yields of green fodder are presented in Table V.

TABLE V
Yield of Senji in different plots

Sl. No.	Depth of water-table (in feet)	Rainfall (in inches)	Total of rainfall and surface irrigation (in inches)	Yield of green fodder (in lb./acre)
1	1	0.74	1.74	26291
2	2	0.74	1.74	29766
3	3	0.74	4.74	29106
4	4	0.74	4.74	27792
5	4-9	0.74	4.74	29766

A glance at the above table shows that *Senji* can be grown successfully in the water-logged areas, having water-table even upto 1 ft. depth. *Senji* is a green manuring crop. It will add nitrogenous material to the soil. Sen² found 2.5% nitrogen in *Senji*. It would also reduce gradually the alkalinity as reported by Lander.¹ *Senji* was used as a green manure in the alkaline plot. This treatment rendered the land capable of bearing an average wheat crop and the analysis showed that the exchangeable calcium had increased. Therefore a rotation of *Senji* in the crop patterns of water-logged alkali soils would greatly ameliorate them by lowering the alkalinity and increasing the nutrient equilibrium.

Sugarcane.—The next crop sown was sugarcane Co. 290.9, Co. 453,

Initial irrigations of 2 in. and 6.0 in. were applied to the plots No. 4 and 5. respectively. The other plots did not receive any irrigation. The total rainfall during the entire period was 28.17 in. No further surface irrigation was given to any plot. The crop fared well in practically all the plots. The respective yields of sugarcane in different plots are given in Table VI.

TABLE VI
Yield of sugarcane in different plots

Sl. No.	Depth of water-table (in feet)	Rainfall (in inches)	Total of rainfall and surface irrigation (in inches)	Yield (in tons/acre)	
1	1.0	28.17	28.17	Co. 290 Co. 453	81.6 93.7
2	2.0	28.17	28.17	Co. 290 Co. 453	91.2 105.3
3	3.0	28.17	28.17	Co. 290 Co. 453	83.8 97.5
4	4.0	28.17	30.17	Co. 290 Co. 453	81.6 94.0
5	4.9	28.17	34.17	Co. 290 Co. 453	61.1 87.6

A moment's reflection at the above Table VI shows clearly that the sugarcane crop can be safely grown in areas having water-table even upto 1 ft. depth. No extra irrigation needs to be given except in areas, where the water-table is below 3.0 ft. depth. In such areas only initial irrigation may be given.

If there is drought then surface irrigation will have to be resorted to especially in those areas where the water-table is below 3.0 ft. depth.

Summing up the above discussion, it is very obligatory that in water-logged areas, the normal canal water allowance should be considerably reduced. The restriction of water allowance will help in checking further rise of water-table. The water saved by this reduction of water allowance can be used for extending irrigation to other areas.

SUMMARY

The paper may be briefly summarised as follows:

Semi-field experiments were conducted in plots, measuring 9 ft. × 5 ft., having pucca floor at 1 ft., 2 ft., 3 ft., 4 ft. and 4 ft. 9 in. depth. From the results of experiments on wheat, cotton, Senji (*Mellotus parviflora*) and sugarcane, the following broad conclusions were drawn:

(a) Wheat did fairly well upto 2.0 ft. of water-table, only one irrigation apart from initial irrigation was given to 4 ft. and 4 ft. 9 in. water-table plots. If another irrigation would have been given to 4 ft. 9 in. depth plot, the yields would have been better.

(b) Cotton did not fair well in plots having water-table upto 3 ft. depth and even the crop was not normal at 4 ft. depth. The crop presented normal growth at 4 ft. 9 in. depth.

(c) Senji was successfully in all the plots.

(d) Two varieties of sugarcane Co. 290 and Co. 453 were tried in all the plots. The yield of Co. 453 was comparatively better than Co. 290. Co. 290 being a soft variety sugarcane did sufficiently well in all the plots. But the best crop was in plots having water-table at 2 ft. and 3 ft. depths.

No extra irrigation was needed in any plot except in Nos. 4 and 5 where 3 in. and 6 in. irrigation were given.

(e) A rotation of green manuring in water-logged areas considerably helps in maintaining the nutrient equilibrium of soil.

(f) A network of drains should be provided in water-logged areas for removing the run off.

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ROLE OF ENERGY-RICH MATERIALS AS CONSERVATORS IN PRESENCE OF NITROGENOUS FERTILIZERS IN SAGAR SOIL

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Read at the Silver Jubilee Session on 28th December 1955,
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It is well known that nitrogen fixed or added as manure, does not remain for a long time in soil under ordinary circumstances. The loss of added nitrogen from the soil has been found to be more with synthetic nitrogenous fertilizers than with natural manures. Researches on soil nitrogen all over world have shown that heavy losses of nitrogen from soil take place when conditions for oxidation are favourable.

Dhar and Pant¹ have found considerable losses of nitrogen in soils from the decomposition of urea and gelatin in complete absence of micro-organisms. Dhar and Mukherjee² have reported that if the circumstances are favourable for oxidation, nitrogen loss takes place even under sterile conditions. Kapoor³ has reported losses of nitrogen from solution of urea during nitrification. The loss has been found to increase with temperature.

The arable soils, if allowed to revert either to a natural forest or to pasture, gradually gain nitrogen and organic matter. Hall⁴ found 0.037% increase of nitrogen after 22 years at Rothamsted by allowing the plots to revert to natural conditions.

Dhar and Mukherjee⁵ have found that sterilized soils fix much nitrogen when exposed to light. Pant, Kapoor and Chatterjee, etc., have found that energy-rich materials like cellulose, green leaves, etc., fix nitrogen in soil. The decrease in losses of nitrogen in urea, hippuric acid, gelatin and ammonium salts have been observed by Dhar's School, when carbonaceous materials are added to the soil. Subrahmanyam⁶ has observed that addition of cellulosic materials is fairly effective in checking the loss of nitrogen following additions of ammonium fertilizers to soil, specially under Indian climatic conditions. Dhar and Mukherjee⁷ have shown beneficial results by adding molasses to fields to which ammonium sulphate is previously added.

In order to throw more light on the problem I have undertaken the study of the extent of decomposition of urea in Sagar soil and the retardation in the loss of fertilizers by adding wheat-straw, lignite, molasses and gur to soil to which urea is previously added.

EXPERIMENTAL

Powdered soil was taken in glass vessels along with urea and energy-rich materials (molasses, gur, wheat-straw and lignite) and exposed regularly for about 8 hours every day. The sets were periodically analysed. The moisture content was

kept almost constant (about 10% of the soil). Mean temperature during exposure was 37° C. The total carbon,⁸ total nitrogen,⁹ ammoniacal-nitrogen and nitrate nitrogen¹⁰ were carried out by standard methods.

<i>Analysis of soil</i>					%
Total carbon	1.133
Total nitrogen	0.203
Ammoniacal nitrogen	0.003
Nitrate nitrogen	0.004
Moisture content at 100-05°	2.056
Loss on ignition	6.125
Silica	36.013
Calcium (CaO)	3.341
Phosphorus (P ₂ O ₅)	0.401

TABLE I
100 gm. soil + 0.6613 gm. urea

Exposure (days)	Organic carbon (%)	Carbon oxidised (%)	Total nitrogen (%)	Ammoniacal nitrogen (%)	Nitrate nitrogen (%)	Nitrogen loss (%)
0	1.133	..	0.511	0.003	0.004	..
15	1.108	0.025	0.492	0.044	0.014	6.26
30	0.999	0.009	0.469	0.031	0.020	13.65
60	0.980	0.019	0.432	0.029	0.022	25.82
80	0.972	0.008	0.411	0.027	0.023	32.38
100	0.954	0.018	0.387	0.020	0.023	40.17
150	0.928	0.026	0.365	0.013	0.029	47.29
180	0.915	0.013	0.348	0.010	0.031	53.05
210	0.908	0.007	0.335	0.009	0.032	57.12

TABLE II

100 gm. soil + 0.6613 gm. urea + 0.6613 gm. wheat-straw

Exposure (days)	Organic carbon (%)	Carbon oxidised (%)	Total nitrogen (%)	Ammoniacal nitrogen (%)	Nitrate nitrogen (%)	Nitrogen loss (%)
0	1.315	..	1.530	0.002	0.004	..
15	1.246	0.069	1.492	0.024	0.020	3.00
30	1.234	0.081	1.461	0.030	0.021	5.13
60	1.171	0.144	1.396	0.028	0.027	10.08
80	1.127	0.188	1.337	0.021	0.029	14.54
100	1.075	0.240	1.276	0.020	0.029	19.12
150	1.034	0.281	1.195	0.019	0.030	25.24
180	0.968	0.347	1.181	0.018	0.031	26.29
210	0.939	0.375	1.168	0.008	0.030	27.28

TABLE III

100 gm. soil + 0.6613 gm. urea + 0.6613 gm. lignite

Exposure (days)	Organic carbon (%)	Carbon oxidised (%)	Total nitrogen (%)	Ammoniacal nitrogen (%)	Nitrate nitrogen (%)	Nitrogen loss (%)
0	1.295	..	1.534	0.003	0.004	..
15	1.276	0.019	1.441	0.022	0.022	3.24
30	1.256	0.240	1.459	0.029	0.040	5.59
60	1.213	0.082	1.376	0.018	0.027	11.73
80	1.174	0.121	1.316	0.017	0.026	16.32
100	1.136	0.159	1.256	0.010	0.025	20.84
150	1.103	0.192	1.187	0.009	0.019	26.03
180	1.091	0.204	1.175	0.008	0.018	26.97
210	1.071	0.225	1.164	0.008	0.015	27.82

TABLE IV

100 gm. soil + 0.6613 gm. urea + 0.6613 gm. gur

Exposure (days)	Organic carbon (%)	Carbon oxidised (%)	Total nitrogen (%)	Ammoniacal nitrogen (%)	Nitrate nitrogen (%)	Nitrogen loss (%)
0	1.254	..	1.533	0.003	0.004	..
15	1.183	0.071	1.507	0.038	0.015	1.93
30	1.172	0.082	1.487	0.034	0.018	3.45
60	1.097	0.157	1.437	0.031	0.022	7.22
80	1.059	0.195	1.394	0.030	0.023	10.46
100	0.996	0.258	1.359	0.021	0.026	12.93
150	0.962	0.292	1.310	0.021	0.032	16.76
180	0.890	0.364	1.294	0.012	0.028	18.00
210	0.867	0.387	1.281	0.010	0.020	18.96

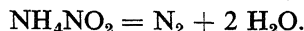
TABLE V

100 gm. soil + 0.6613 gm. urea + 0.6613 gm. Molasses

Exposure (days)	Organic carbon (%)	Carbon oxidised (%)	Total nitrogen (%)	Ammoniacal nitrogen (%)	Nitrate nitrogen (%)	Nitrogen loss (%)
0	1.481	..	1.525	0.003	0.004	..
15	1.420	0.061	1.497	0.044	0.015	2.12
30	1.400	0.081	1.470	0.038	0.020	4.16
60	1.348	0.133	1.401	0.032	0.022	9.28
80	1.296	0.185	1.335	0.030	0.024	13.63
100	1.245	0.236	1.322	0.027	0.030	15.35
150	1.201	0.280	1.285	0.020	0.030	18.14
180	1.149	0.332	1.275	0.013	0.021	18.89
210	1.118	0.363	1.271	0.010	0.011	19.24

DISCUSSION

Dhar and co-workers,¹¹ for a number of years carried out extensive researches on slow oxidation of ammonium salts and other nitrogenous compounds in soil in presence of air. They observed that about 60% of the added nitrogen was lost due to oxidation by light and temperature. Russell¹² also reported a recovery of only 40% of added nitrogen in form of ammonium salts. Dhar and Narayanan¹³ suggest that during the oxidation of the nitrogenous fertilizer in soil, the free ammonium ions which in their turn may further be oxidised to nitrate ions or may form a highly unstable intermediate compound, ammonium nitrite, which decomposes to give rise to more of gaseous nitrogen as per equation:



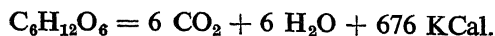
The whole mechanism of nitrogen transformation in soil has been generally accepted as taking place in the following manner:

Protein or other organic matter—amino acids—O₂ ammonia—O₂ nitrate—O₂ nitrite.¹³

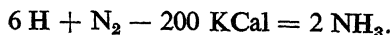
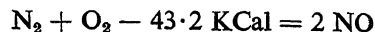
Hence there is always the possibility of ammonia and nitrate existing side by side, which therefore together form ammonia nitrate, most of which decomposes to give free nitrogen gas. This loss is greater when larger amounts of fertilizers are used in the form of ammonium salts, for then the chance for the presence of larger quantities of ammonium nitrite in the stage of oxidation of ammonium salts is all the more prevailing.

It is clear therefore that when ammonium salts or urea are added to soil, the first product formed is ammonia which in its turn is converted into its oxidised products like nitrite and nitrate. Under these conditions at ordinary temperature, the whole of ammonia cannot be converted into oxidised products quickly; and hence ammonium ions and occasionally free ammonia has to co-exist with nitrite ions or nitrous acid, nitrate ions and/or nitric acid, causing decomposition of urea with evolution of nitrogen gas.

It has already been stated that addition of carbonaceous materials retards considerably the loss of nitrogen due to the decomposition of the added fertilizer, causing thereby a remarkable retardation in the loss of nitrogen of urea in presence of wheat-straw, lignite, gur and molasses in soil. The energy-rich materials undergo oxidation in presence of sunlight and a large amount of energy is liberated.¹⁵ Thus,



This liberated energy is much more than needed for fixation of atmospheric nitrogen by either of the following mechanisms.



Thus considerable nitrogen is fixed as ammonia which gets dissolved in the soil. The carbon of the energy-rich materials, therefore when added to soil, retard the velocity of oxidation of ammonium ions to nitrate, thus consequently decreasing the loss of nitrogen.

Our observations lead to the conclusion that when urea is added to soil and exposed to sunlight for a period of 210 days a loss of nitrogen to the extent of 57.12% takes place in the system. This is, however, retarded to 27.28%, 27.82%, 18.96% and 19.24% when wheat-straw, lignite, gur and molasses respectively are added to the soil containing urea and exposed to sunlight. The rate of the loss of the nitrogen from the system and also the efficiency of the energy-rich materials in retarding the said loss are found to be greater at the initial stages and they are slowed down later.

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